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The Encapsulation of Organic Molecules and Enzymes in Sol-Gel Glasses: Novel Photoactive, Optical, Sensing and Bioactive Materials. A Review

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The ability to trap organic and inorganic molecules in inorganic oxides through the sol-gel process, first introduced in 1984, opened the road for a whole new class of materials and to intensive activity in many laboratories. The types of materials and their applications are reviewed. These include photocatalysts for redox reactions; photochromic materials and other information recording materials; filters and light-guides; fluorescent, phosphorescent and dye-laser materials; a variety of chemical sensors; and bioactive (enzymatic) glasses.

1. Background

1.1 The Sol-Gel Process

One of the major revolutions in modern materials science has been the advent of a novel synthetic route for the preparation of glasses, known as the "sol-gel processes". Reference 1 is an excellent, comprehensive text on this topic, and no attempt can be made in this introductory section to even scratch the surface of this huge field. Consequently, following are only some of the very basic concepts needed for understanding this review, and the interested reader is referred to (1) for further details.

The basic idea of the sol-gel process is to split the glass preparation into two main stages: The first stage is a room temperature polymerization of a suitable monomer leading to a porous glass, usually an inorganic glass. The second stage is the closure of the pores at elevated temperatures (several hundreds degrees), forming the final glass. Two of the main advantages of this technique are: First, the overall energy-saving due to the lower temperatures utilized; and second, the ability to achieve unconventional glass compositions by a suitable choice of a mixture of monomers. Such compositions may be unobtainable through the classical methods due to phase-separation upon cooling.

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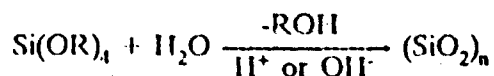
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Most of the glasses described in this review are porous silica glasses, (and their modifications) obtained by the general reaction (unbalanced):



in which $\text{R} = \text{CH}_3$ or CH_3CH_2 . We concentrate here on the porous materials obtained from the first stage of the sol-gel process. Other glasses reported in this review are alumina and titania, which are obtained by similar reactions. Reversed-phase porous glasses are also mentioned below. These are obtained by polymerization of monomers of the type $\text{R}^*\text{Si(OR)}_3$ in which R^* is a non-polymerizable substituent on the silicon, such as a long-chain alkyl. Finally, composite glasses in which organic polymeric molecules are incorporated in the inorganic glass, will also be mentioned.

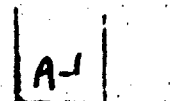
Typical properties of the porous glasses are: a) high surface area (several hundreds of m^2/gr); b) low densities (around 1 gr/ml); c) narrow pores (around ten nm), and consequently; d) complete transparency to visible light and to the near u.v.; e) the ability to obtain any desired shape, including thin films; f) good chemical, photochemical and thermal stability. The brittleness of these glasses has been a major problem, but this is gradually being solved in various laboratories by modification of the synthetic procedures and by the use of proper additives (1).

1.2 The Trapping of Organic Molecules in Inorganic Glasses: A Novel Class of Materials.

From the point of view of organic and bioorganic chemistry the most important application of the sol-gel process has been the ability, for the first time in the four-thousand years history of glass technology, to dope (oxide) glasses with organic molecules. Until recently doping was limited to salts and oxide-additives, which can withstand the high temperatures involved in the melting procedures. Since the vast majority of the $7 \cdot 10^6$ compounds known today are organic or bioorganic, the new horizons that have become open now, are limited only by imagination. The basic idea is simple: Adding a solution of the desired dopant to the starting polymerizing system, and stopping the glass preparation at the dry xerogel stage. Surprising as this may be, that simple idea is quite recent: It was first described in 1983 in a patent application (2) and a year later in *J. Phys. Chem.* (3). It is, perhaps, less surprising that this glass-doping idea has since then expanded quite rapidly. Most of the applications developed at first concentrated on the preparation of materials for optical and photophysical uses, a trend which is still going on strongly. However, a new class of materials has been recently developed based on the doping idea, namely, chemically active sol-gel glasses. Catalysts, sensors and the exciting group of bioactive materials, are some examples to be discussed below.

Some of the properties and advantages of the doped sol-gel glasses which are of use for the applications reviewed here are the following:

1. The glasses are stable thermally, chemically and photochemically,



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especially when compared to plastic matrices, which were the only materials used so far for encapsulation of organic molecules.

2. The encapsulated molecules are well protected again, much better than in plastics. For instance, their photodegradation rate is smaller, compared to solution (3).

3. The glasses are transparent well into the u.v., allowing a whole array of photochemical, photophysical and optical applications.

4. The trapped molecules are either non-leachable, or, in some cases, leachable over a very long time period.

5. A sub-population of the non-leachable molecules is trapped close enough to the pore-surface, to be able to interact with substrate molecules contained in the pore space. It is this portion of trapped-yet-exposed molecules which allows the development of chemically active sol-gel glasses.

We begin our review with the class of reactive glasses. We then proceed to bioactive glasses obtained by trapping of enzymes (Section 3). Section 4 describes the variety of applications that were developed in photophysics and optics. Spectroscopic properties of trapped molecules were used for the study of the properties of the glass cage and for follow-up of the structural changes that occur along the polymerization stages, from the sol to the xerogel. These studies are then outlined in Section 5.

2. Chemically Reactive Organically Doped Sol-Gel Glasses

2.1 Photoreactive Sol-Gel Glasses.

The ability to achieve photochemical interactions with organic substrates or catalysts trapped in sol-gel glasses was demonstrated by several research groups. First, we briefly review these reports and then proceed to a description, in some detail, of sol-gel systems exhibiting photoinduced charge separation.

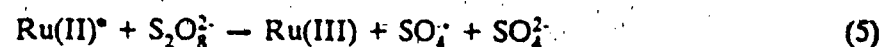
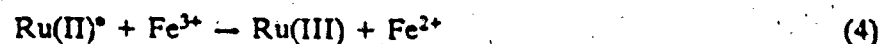
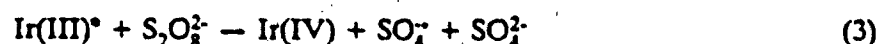
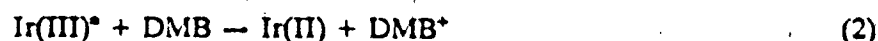
Studies aiming at establishing the mechanism leading to and controlling the size of metal oxide and metal particles in sol-gel glasses were performed by Modes and Lianos (4). These authors examined the quenching of the luminescence of $\text{Ru}(\text{bpy})_3^{2+}$ by methylviologen in SiO_2 gels prepared by adding both reagents to the initial polymerizing solution of tetramethoxysilane under a variety of (e.g., pH) conditions. It was shown that in the gel the lumophore and quencher are aggregated as molecular clusters and that glasses, in which $\text{Ru}(\text{bpy})_3^{2+}$ is replaced by cadmium or lead salts, form CdS and PbS particles upon exposure to H_2S .

Quenching experiments were carried out with $\text{Ru}(\text{bpy})_3^{2+}$ and with a pyrene quaternary ammonium salt doped within silica gels prepared from $\text{Na}_2\text{SiO}_3 \cdot \text{H}_2\text{O}$ by acid ion-exchange (5a). Quenching was effective for quenchers co-trapped with the fluorophore. However, exposure of the gels to atmospheric oxygen did not result in any change in excited state lifetimes indicating that the latter are inaccessible to atmospheric O_2 . A different behaviour was observed in the case of pyrene-doped (crystalline or amorphous) TiO_2 powders prepared from titanium tetrapropoxide (5b). Pyrene was found to occupy sites which were readily accessible to water and ionic quenchers. However,

photoproducts which are normally associated with excited pyrene were not observed in TiO_2 particles, a fact which was attributed to the close proximity of the products, which are formed under conditions of limited diffusion, thus enhancing back reaction.

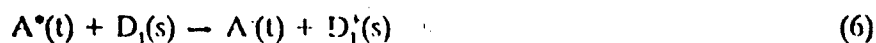
Additional evidence showing that a substantial fraction of the trapped molecules do have access to the intra-pore volume was based on the observation that a trapped organic iridium complex $[\text{Ir}(\text{bpy})_2(\text{C}_3\text{N}^+)\text{bpy}]^{3+}$ (abbreviated as Ir(III)) is effectively quenched by dimethoxybenzene (DMB) dissolved in the liquid pore network of the gel matrix. Similarly, Pouxviel et al. reported (6) that pyranine (a hydroxyl pyrene derivative, ROH) molecules in a sol-gel matrix are sensitive to the composition of the intra-pore solution. The presence of water increases the polarity of the pyranine environment, enhancing dissociation of the excited state as monitored by the emission of the resulting RO^{\bullet} moiety.

The observation that the luminescence of a substantial fraction of a glass-entrapped Ir(III) complex undergoes quenching by DMB, not only opened the door to the use of doped sol-gel glasses as chemical sensors, but also suggested applications in photocatalysis (7). The process was studied recently by Slama-Schwok et al. (8) for several donor-acceptor pairs: pyrene* (Py^*)/ MV^{2+} , Ir*(III)/DMB, Ir(III)*/ $\text{S}_2\text{O}_8^{2-}$, Ru(II)*/ Fe^{3+} , and Ru(II)*/ $\text{S}_2\text{O}_8^{2-}$, where the asterisk denotes the excited, glass-trapped sensitizer and the counterpart molecule or ion is present in the intra-pore liquid phase. (Ru(II) denotes the $[\text{Ru}(\text{bpy})_3]^{2+}$ complex). By applying laser photolysis methods it was shown that photoinduced electron transfer between the donor and acceptor located in the two phases, appears to be a general phenomenon, independent of the nature of the specific pair. Thus, in all cases a substantial fraction of the photosensitizer population was quenched, leading to the corresponding charge-separated radical ion pairs:



By studying the concentration dependence of both quenching efficiency and quencher adsorption, it was concluded that the predominant mechanism involves long-range electron-transfer (LRET) from, or to, an adsorbed solute molecule and a photosensitizer embedded in the glass. The LRET mechanism is responsible for non-exponential quenching rates which reflect a distribution of distances between donor and acceptor.

The photoinduced electron-transfer process (2) may be generalized as

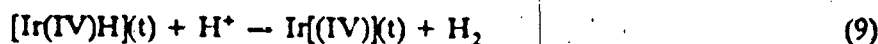


where A and D_1 represent electron acceptors and donors, respectively, while (t) and (s) represent glass-trapped and intra-pore solute species respectively. Note that the situation is reversed in (1) where D_1^* is glass-entrapped and A is in the solution. A striking observation in the cases of (1) and (2) was the extremely long life of the radical ion-pair. In both cases the corresponding back reaction, e.g.,



was found to be slower, by 4-5 orders of magnitude with respect to the same (diffusion-controlled) process in homogeneous solutions. Such a retardation is among the highest achieved in any heterogeneous photochemical system (9,10). The effect may be rationalized in terms of the same LRET mechanism and as suggested for the forward quenching reaction (6): A distribution of separations between $D_1(s)$ adsorbed at the interface and $A^*(t)$ trapped at different depths from the surface, will yield a distribution of separations between surface $D_1^*(s)$ and trapped $A(t)$. An important factor in retarding the back reaction (7) is associated with the adsorption of $D_1^*(s)$ at sites which are distant from a trapped $A(t)$ counterpart (7).

These observations are relevant to the general problem of photochemical light-energy conversion. Thus, retardation of the back electron-transfer reaction is a major prerequisite in any artificial photosynthetic system (9). In the specific $Ir(II)^*/DMB$ (sol-gel) system the absence of an effective back reaction allows (at acidic pH) the strong reductant $Ir(II)$ to react with water yielding molecular H_2 according to (7):



Since in the above (binary) system DMB^+ is incapable of completing the cycle by evolving O_2 from water, the net result is the generation of H_2 at the expense of DMB.

An ideal photosynthetic device is one in which the back reaction is totally inhibited by complete immobilization of both A^* and D_1^* . This will, in principle, allow one to carry out catalyzed reactions of the two species with, e.g., H_2O leading to the generation of both O_2 and H_2 .

An important approach in reaching this goal is to overcome the difficulties encountered in two-component (A , D_1) systems by applying three (or more) molecules with appropriate redox properties, where, e.g., the primary donor D_1 acts as an intermediate charge-carrier ("shuttler") to a secondary donor D_2 (10,11). This was achieved recently in a sol-gel glass by inhibiting the back reaction in a system where A and D_2 (and thus also A^* and D_2^*) are completely immobilized by trapping in the three dimensional network of the sol-gel glass.

matrix (12). Accordingly, reaction (7) is inhibited by (10):



in which the mobile charge carrier D_1^+ in the intra-pore phase transfers its positive charge to the immobilized secondary donor $D_2(t)$. The final products are $A(t)$ and $D_2^+(t)$, both immobilized in the glass matrix. The particular (ternary) redox system employed was: $A_1^+(t) = \text{Ir(III)}$, $D_1(s) = \text{DMB}$, $D_2(t) = \text{Ru(II)}$. The final primary photoproducts were Ir(IV) and H_2 (generated via (8) and (9)) and Ru(III) generated via (10) (i.e., via $\text{DMB}^+ + \text{Ru(II)} \rightarrow \text{DMB} + \text{Ru(III)}$).

The ultimate goal is that of generating a long-lived radical pair where the reactions of both partners to yield useful fuels such as H_2 and O_2 would be controlled by means of appropriate catalysts. The prospective of applying doped sol-gel glasses to the photochemical conversion of light energy is especially appealing also in view of the inertness of the inorganic matrix. This offers considerable advances over microenvironments such as membranes, vesicles and organic polymers (9,10) where irreversible side reactions with the matrix decrease the efficiency and shorten the lifetime of feasible devices.

2.2 Chemical Sensors.

The observations described in section 2.1 suggested that suitable analytical reagents trapped in porous sol-gel glasses may be used for the preparation of a variety of chemical sensing materials. It was indeed shown that after condensation, drying and thoroughly washing with appropriate solvents, one obtains an almost non-leachable glass doped with an appropriate reagent which is sensitive to solutes in the adjacent liquid phase (13a). Characteristic examples are classical reactions of cations and anions resulting in the coloration of the reagent-doped glass (rod or disc), e.g., Fe^{2+}/α -phenanthroline, $\text{Ni}^{2+}/\text{dimethylglyoxime}$, Cu^{2+}/α -benzoinoxime, $\text{SO}_4^{2-}/\text{sodium rhodizonate} + \text{BaF}_2$, etc. Very high sensitivities, e.g., 100 ppt Fe^{+2} in water, were recorded (13b). Due to the optical properties of the glass matrix the tests may be carried out visually as well as spectrophotometrically.

The exact mechanism of reagent trapping and exposure is still unclear. Trapping may be due to strong adsorption interactions at the glass-liquid interface, which are due to the morphology of the surface. It is also important to note that some of the color tests are associated with complex formation, with a reagent: metal ion ratio of 2:1 or even 3:1. This implies that at least some of the reagents are trapped as aggregates which are accessible to the metal analyte. Moreover, it is implied that tumbling of the reagent, sufficient to achieve appropriate intramolecular orientations in the complex, does take place. The latter hypothesis is indirectly supported by the e.s.r. experiments of Ikoma et al (14), showing that polyamine copper(II) complexes in wet alumina gels are almost free in tumbling motion. In spite of these open mechanistic questions, it is evident that doped sol-gel glasses may effectively function as sensors to inorganic and organic solutes. This also applies to their use as

reversible pH indicators, based on the inclusion of appropriate indicators in the starting sol-gel reaction mixture (13,15). Along the same lines, a glass-entrapped pH-sensitive dye was recently applied as sensor to atmospheric ammonia or acid (16).

2.3 Trapping of Catalytic Metal and Oxide Particles.

Although the topic of this review is the trapping of organic molecules in sol-gel glasses, we believe it is important to include here also a brief comment on the application of the sol-gel method for the preparation of trapped inorganic catalytic particles, because of the major importance of this topic to industrial catalytic processes.

The use of oxides such as silica, alumina and titania as supports for dispersed metal and metal-oxide catalysts has been at the focus of catalytic chemistry for quite some time. Catalyst deposition methods such as metal evaporation (17), mechanical admission (18), ion exchange (followed by thermal decomposition of the surface-bound salt) (19,20), or impregnation (21), all involve the dispersion of catalyst particles on a predetermined surface of a porous solid or of colloidal particles. With the purpose of providing a novel technology, leading to a more convenient catalyst with better controlled dispersion, methods were devised, based on the incorporation of the catalyst particles in the bulk of porous silica or alumina matrices through sol-gel methods. The basic approach is to hydrolyze a mixed solution of the alkoxy compound in the presence of a soluble salt of the metal or oxide catalyst, leading to an active gel containing the catalyst particles (18).

A typical example involves an iron particle catalyst prepared by drying and calcination of a gel obtained by hydrolysis of a mixed solution of ethylsilicate and iron (III) nitrate (21). The Fe particle distribution (5-20 nm), determined by EXAFS and IR spectroscopy, was found to be better controlled than, e.g., by the alternative impregnation (i.e., adsorption) methods. Inorganic aerogels with large pore volumes and high surface areas prepared by the autoclave method, were later used for the incorporation of a variety of metal or metal oxide catalysts (21). Systems investigated included NiO/alumina and NiO/alumina-silica (isobutylene oxidation), Ni/alumina (ethylbenzene hydrogenolysis), Cu/alumina (cyclopentadiene hydrogenation) and Ni/MoO₃. Relatively high selectivities were reported for the various catalysts and related reactions.

Studies of SiO₂-supported catalysts indicated advantages of high purity support which is free of possible poisons (22). A comparison of the self-poisoning of such metal (e.g., Ru) catalyst, with one prepared by the classical impregnation method, was performed by Lopez et al. (23). No systematic advantages or disadvantages of the sol-gel method (with respect to catalyst deactivation) were found for gas-phase hydrogenation reactions. It appears, however, that sol-gel glass-entrapped nickel-alumina catalysts exhibit a higher selectivity in the liquid phase hydrogenation of 1,3- and 1,5-cyclooctadiene and methyl linoleate than with Raney nickel or impregnated nickel-alumina catalysts (24). A relatively lower activity of the sol-gel catalyst

was attributed to the tendency of the nickel particles to be encapsulated within the alumina matrix.

The existence of the doping salts in the form of molecular aggregates was directly demonstrated using e.s.r. methods by Ikoma et al. (20) in the case of polyamine copper(II) complexes in silica (sol-gel) glasses. The complexes were thermally decomposed to copper(II) oxide and copper metal particles of the order of several hundreds Å. Similar experiments were carried out in alumina gels prepared by the sol-gel technique (14). The important aspect of Ikoma's et al. work, from the point of view of this review, is the demonstration of the ability to obtain inorganic catalysts by doping with organic precursors, such as organic metal complexes and metal organic salts.

Very recently, Hardee et al. described the co-polymerization of n-triethoxysilyl-1,2-bis(diphenylphosphino)benzene with silicic acid, as a precursor for a rhodium catalyst for alkene hydroformylation and methanol homologation, but only low catalytic activity was observed (24a).

3. Enzymes Immobilized in Sol-Gel Glass Matrices: Bioactive Glasses

In this section we describe what we believe to be the first successful trapping of cell-free enzymes in sol-gel glasses.

Attachment of biologically active molecules to an insoluble matrix is an essential, and in some instances an indispensable step in the development of biocatalysts. Immobilization allows reuse of enzymes, protects them from harsh external conditions, from microbial contamination, and prolongs their useful lifetime. A large number of existing immobilization techniques reflects the complexity of the biological material and the variety of its applications. Simple inexpensive general techniques, resulting in stable and active enzyme catalysts are yet in demand (25).

A good carrier matrix should provide the enzyme with mechanical and chemical stability combined with large, accessible and hydrophilic surface. Porous glasses and hydrophilic silicas conform well with these requirements. However, the bonding of an enzyme to these matrices requires tedious chemical derivatization procedures.

The sol-gel glass technology provides the opportunity of bonding enzymes to glass using the most generally applicable immobilization procedure, namely, a simple entrapment. The main shortcoming of the entrapment technique as applied to a variety of natural or synthetic polymers is the loss of the enzyme by leakage through a nonuniform net of polymer molecule chains. In contrast, enzymes entrapped in sol-gel glasses are not removed from the catalyst particles even following extensive washing with salt or with slightly alkaline solutions. Only at pH above 10, some leaching of both the silicate and the protein was observed.

Diffusion of protein within the glass matrix is very limited. Thus, glasses were prepared containing brightly colored occlusions of hemoglobin aggregates in a clear glass. This glass was then immersed in water for as long as one month. No visible spreading of the red hemoglobin was detectable. The narrow

uniform pore range of sol-gel glass (3-10 nm) may account for this phenomenon (30).

For an entrapment of an enzyme, the latter has to be added in water solution to a mixture of monomers at the onset of polymerization or later during the gel formation. When an enzyme is entrapped in a gel formed by a water-soluble polymer (gelatin, alginate, polyacrylamide) at low temperatures, denaturing conditions can be easily avoided. Use of organic solvents in the process of sol-gel formation may cause denaturation of the enzyme. Thus, Carturan et al. (26) have been able to demonstrate invertase activity in aggregates of whole yeast cells trapped in thin sol-gel films, whereas films with cell-free invertase were devoid of activity. This inactivation of the cell-free enzyme may be caused by denaturation. The enzyme in whole yeast cells was, probably, protected from the denaturing effect of alcohol by protein-rich cytoplasm. It seems, that a certain degree of protein aggregate formation is important for protection of enzymatic activity during immobilization (30). It is hardly accidental that for a successful immobilization of the cell-free alkaline phosphatase (27,28), it was essential to work at conditions near the precipitation of the enzyme. At the volume ratio of tetramethoxy orthosilicate (TMOS) methanolic solution to water 12:1 (compared to 6:1 used by Carturan et al. (26)) alkaline phosphatase actually precipitated during gel formation. Light scattering by protein aggregates (see Fig. 1 in ref. 27) was observed both before and after the hydration of the alkaline phosphatase glass. This aggregation or precipitation did not impair relatively high yield of enzymatic activity (about 30%).

Milder and less denaturing precipitating agents, such as polyethylene glycols were more effective in preservation of enzyme activity than lower alcohols, even in the absence of visible precipitation. Several enzymes have, thus, been successfully immobilized. The accumulated data do yet allow only preliminary conclusions concerning the influence of various additives and of gelation conditions. High yields of trypsin activity (50-60%) were obtained by mixing a slightly acidic enzyme solution in water containing PEG 6000 (3%) with an equal volume of TMOS at room temperature. The opaque mixture became homogeneous in 5-10 minutes, as TMOS was hydrolyzed, and congealed in about an hour. Clear monolithic glasses form after drying at 30-37°C for a week. These glasses sometimes crack during hydration (30).

Trypsin, aspartase, peroxidase and other enzymes have been entrapped in heterogeneous polymerization systems containing enzyme solution in water (0.2 ml/ml TMOS) and PEG 400 (0.6-0.8 ml/ml TMOS). Such mixtures separate into two phases: the upper (organic) phase contains TMOS saturated with PEG and traces of water, while the lower contains mostly PEG and water with little TMOS. Gel formation starts at the interface by initial hydrolysis of TMOS. Hydrophilic products of hydrolysis and polymerization undergo partition predominantly into the lower phase, where the gelation occurs. Protein aggregates or other relatively hydrophilic material (e.g. microbial cells) were always found only in the lower phase. Thus, during the formation of glass the proteins are not in contact with denaturing hydrophobic phase. After 3-4 h at

4°C, a solid glass-like material forms in the lower phase creating a diffusional barrier for further partitioning of precursors. The upper phase is then removed and the resulting homogeneous protein-containing sol-gel is dried. Cell free aspartase immobilized using this technique, resulted in highly active enzymatic catalyst, with a yield of enzyme activity similar to that of an industrial enzyme described by Chibata et al. (29).

In general, recovery of enzymes in polymerization mixtures containing methanol was poor. The volume change during the drying in the PEG containing polymerization systems was significantly lower than in methanol-based mixtures, and the latter, usually, shrank to about 10% of their initial volume. We believe that the shear generated during this shrinking may damage protein molecules.

Scanning electron microscope (SEM) pictures of protein-doped glasses show spongy uniform glass, sometimes dotted with protein aggregates. The degree of aggregation depends upon the protein and upon the conditions of precipitation. The recovery of enzyme activity in heavily doped glasses is usually low. We have noticed that the gelation is considerably speeded up by biological material such as proteins or microbial cells, probably leading to a denser, less porous glass.

The analysis of substrate dose-response curves for alkaline phosphatase (ALP), immobilized in methanol-containing polymerization mixture, revealed at least two kinetically different forms of the enzyme (27). The high affinity enzyme component had the Michaelis constant $K_m = 0.8$ mM, which was also measured for the soluble ALP. About 90% of the enzyme activity, however, had an average K_m of 7 mM. The pH maximum of immobilized ALP was about one pH unit higher than for the native enzyme. However, trypsin and acid phosphatase, prepared in the presence of PEG, demonstrated single form kinetics with K_m close to that of the soluble trypsin (30).

Sol-gel-immobilized trypsin activity towards small substrates was not inhibited either by soy bean trypsin inhibitor (20.1 kDa). Poly-L-lysine of molecular size above $M_r = 29.6$ kDa had no effect on immobilized trypsin activity, while in the range of $M_r = 15-8.0$ kDa this polymer inhibited the biological glass activity to the same extent, as that of the soluble enzyme. All this indicates effective pore sizes of about 2-4 nm (30).

We have compared the ability of the soluble and of the immobilized enzymes to form a complex with the dye Coomassie blue. This dye binds to the positive charges on protein molecules, and, thus, serves as a general protein surface probe. Coomassie blue binding was well correlated with the activity of the immobilized enzyme (28). This observation signifies equal accessibility of the protein to general and to active site-targeted probes. It could be interpreted as indicating that either enzyme molecules were partitioned in accessible and inaccessible phases, or that a part of a single protein molecule surface was interacting tightly and at random with the sol-gel matrix. The narrow effective pore size pointed out to the latter possibility. The existence of such tight interactions and the strong character of the binding forces could be demonstrated by diffusional limitations of the immobilized enzyme. Thus,

loosely immobilized (e.g. entrapped in a polyacryl amide gel) trypsin can completely degrade itself when left at neutral or slightly basic pH for prolonged periods of time. Various sol-gel immobilized trypsin preparations have lost only 10-30% of their activity when incubated at 30°C overnight at pH 7.5 (28,30). This indicates that most of the trypsin molecules are not able to diffuse within the network of the sol-gel. Trypsin can be reversibly absorbed on sol-gel glass, while retaining its activity. As expected, such physisorption-immobilized trypsin was completely autodigested under the conditions described above (30).

Firm interaction between the immobilized protein molecule and the matrix is a characteristic of covalently bound enzymes, rather than of entrapped ones. In covalently immobilized enzymes a protective effect of the immobilization matrix against thermal inactivation, or the increase in the melting point of protein polypeptide chain, depends on the amount of binding sites per protein molecule (31). Multipoint (3 to 8 points) attachment of the immobilized enzyme results in a considerable increase of the denaturation temperature. Thus, one would expect significant stabilization of sol-gel immobilized enzymes. Indeed, it has been shown that sol-gel immobilized ALP is more stable at 70°C than its soluble form (27). An even more dramatic protective effect was observed in sol-gel immobilized preparations of acid phosphatase, which was an order of magnitude more stable than the soluble enzyme. We believe that during sol-gel formation the matrix and the glass form closely fitted surfaces generating strong binding forces. The high thermal stability of sol-gel glass immobilized enzymes allows to use them at elevated temperatures, thus increasing the reaction rates.

In conclusion of this section: We have demonstrated that the sol-gel method of enzyme immobilization by entrapment of the enzyme in a porous sol-gel glass produces a biocatalyst in a simple generally applicable procedure. A variety of enzymes representing different classes of catalysts have been already successfully immobilized. It has been shown that a large (up to 100%) population of trapped molecules can react with small external substrates through the pore network of the glass. The final product is an air-dried material with a shelf-life of more than six months. That makes it uniquely convenient for shipment and storage. In its hydrated active form, the catalyst is also stable for a very long time. This method of immobilization allows one to obtain highly active catalysts of the desired geometry: Beads, thin layer coating on various supports (such as glass, ceramics, metal), etc.

The sol-gel glass trapping of enzymes with a wide variety of applications are worldwide patent pending.

4. Sol-Gel Glasses for Optical and Spectroscopic Applications

4.1 Light Absorbing Materials

4.1.1. Photochromic glasses. The search for ever better information recording and information processing materials has never ceased in our

information oriented society. A major family of molecules which has served for that purpose are the photochromic compounds (32). These have been used for chemical computer-switches, as signal processors, as reusable information storage media, as microimaging materials, as protective materials against irradiation, as photomasking and photoresisting materials, to mention a few applications. The currently used photochromic glasses are based on a very limited selection of inorganic dopants. However, the ability to trap organic photochromic dyes in the sol-gel glasses, opens the possibility to use the thousands of existing photochromic molecules (32), with the ability to tailor desired properties such as the nature of color change, the activating wavelength, the rates of response to light, the fading rate, etc.

The feasibility of this idea was first demonstrated briefly in reference 33 in which the trapping of the photochromic dye Aberchrome-670 in SiO_2 was described. Difficulties with long-lasting activity of that dye, have shifted the attention to the largest class of photochromic molecules, namely to the spiropyranes. These were used for both a mechanistic study of the sol-gel-xerogel process ((34), see Section 5) and for the preparation of good photochromic materials. Levy et al. have described two types of photochromic materials which are based on trapping of five different spiropyranes in modified SiO_2 matrices (35). One type of matrix was obtained by polymerizing $\text{CH}_3\text{CH}_2\text{Si}(\text{OCH}_2\text{CH}_3)_2$. This results in a network which is less cross-linked than the usual SiO_2 matrix, and which has an a-polar cage comprised of SiCH_2CH_3 groups. The normal photochromic behaviour of the spiropyranes was obtained with these materials, namely colorless glass converted upon irradiation to colored glass. Interestingly, when the sol-gel process was employed with a 4/1 mixture of $\text{Si}(\text{OCH}_3)_4$ /polydimethyl siloxanes (of various molecular weights) materials with reverse photochromism were obtained, namely colored in the dark and fading through the action of light. The difference between the normal and reversed photochromisms in these two types of materials was explained in terms of the differences in the cage properties within which the dye was trapped, namely, low polarity in the former and higher polarity in the latter.

Reversed photochromism of spiropyranes was observed in sol-gel glass obtained from the polymerization of $\text{Si}(\text{OCH}_3)_4$ (34) and also, as reported by Matsui et al. (36) from $\text{Si}(\text{OCH}_2\text{CH}_3)_4$. However, in these unmodified glasses, the photodynamics slow down significantly in the aged glass, due to the high rigidity of the final cage. However, the two organically modified glasses described above provide long lasting materials. The added flexibility of those matrices is the direct cause of that improvement.

Stable photochromic materials were also obtained by encapsulating a number of spiropyranes in aluminosilicate sol-gel glasses (37). These were obtained by the polymerization of $(\text{CH}_3\text{CH}_2\text{CH}_2\text{O})_2\text{Al-O-Si}(\text{OCH}_2\text{CH}_2\text{CH}_3)_3$. The photochromic properties were used for the investigation of this polymerization (Section 5).

4.1.2 Glasses for Hole-Burning and Nonlinear Optics. Another photochemical method with great potential for information recording purposes is the process of hole-burning (38). It is a high-resolution photobleaching technique which can, in principle, store data at a density which is a 1000 fold higher than in present optical disc systems (38). Sol-gel glasses have been studied for these purposes by Tani et al. (39,40) by Locher et al. (41), by Kobayashi et al. (42) and by Tanaka et al. (43). In a typical study (40), 1,4-dihydroxyanthraquinone was used as a dopant for SiO_2 glasses prepared by acidic polymerization of $\text{Si}(\text{OCH}_2\text{CH}_3)_4$. The observed burning yield was $1.2 \cdot 10^{-4}$ which is comparable to yields obtained in organic glasses. An intrinsic holewidth of 0.9 cm^{-1} was obtained at zero burning time. The annealing of the burned hole indicated two types of mechanisms which dominate the temperature dependence of the holewidth: A reversible one which peaks below 27°K and an irreversible one above that temperature.

Tanaka et al. (43) observed efficient hole-burning activity in sol-gel alumina thin films, having in mind wiring applications of these materials.

On a very basic level, it has been demonstrated that the sol-gel technique can be used for the preparation of filters. A large variety of organic molecules can be used for that purpose and this has been demonstrated for SiO_2 films (44) and blocks (39), for $\text{SiO}_2\text{-TiO}_2$ films (44), for Al_2O_3 films (42) and for SiO_2 -poly-methyl methacrylate composite (45). On a more advanced level, since all of the dyes trapped in these studies are asymmetric π -conjugated molecules, many of these glasses can be used in principle for nonlinear optic purposes. These dyes include, e.g., acridines (39), 2-methyl-nitroaniline (45), fluorescein (44,46) and coumarines (46a). Third order nonlinear effects were observed by Knobbe et al. (47) in polyaniline doped SiO_2 -sol/gel films and in coumarins and rhodamines, trapped in originally modified silica sol-gels (48).

4.1.3 Electro-optical Liquid Crystals Sol-Gel Glasses. A most interesting application of sol-gel glasses was suggested recently by Levy, Serna and Oton (49). These authors prepared thin films of reversed-phase silica (obtained from $(\text{CH}_3\text{CH}_2)_3\text{Si}(\text{OCH}_2\text{CH}_3)_3$), in which microdomains of liquid crystals of 4-methoxybenzylidene-4'-n-butylaniline were encapsulated. The drive for that study has been the current major obstacle in the application of liquid crystals for electrooptical devices, namely the lack of suitable materials which have both the required electrooptical properties and can also be prepared in suitable forms such as thin films and monolithic blocks. Microscopy studies showed that the microdomains vary in size from 0.5 to 3 microns. The orientation of these domains was found to be affected by the ethyl groups on the walls of the pores. Transitions between nematic phase and liquid phase were observed by following laser diffraction patterns. Most importantly, it was found that the trapping process does not alter the electrooptical and thermal behaviour of the liquid crystals.

4.2 Light Emitting Materials

4.2.1 Fluorescent and Dye-Laser Materials. Chronologically speaking, fluorescent dyes were both the first organic molecules which have been incorporated in sol-gel glasses (2,3,50) and the most numerous as a class. Examples include rhodamine 6G (3,39,42,44,51,52), naphthazir (39), acridines (39) coumarines (39,42), rhodamine B (39,42,44,45,53,54) ruthenium complexes (44,55,56), fluorescein (44,46,57), crystal violet (42,44), malachite green (44), oxazines (16,42,44), 4-methylembelliferone (45), nile-blue (42), porphyrines (42), resorufin (42), cresyl-violet (42) and phthalocyanine (42). Additional ones are mentioned in Sections 2 and 5.

Many of these glass preparations were aimed at studying the properties of the glass cage to which we return in Section 5. Some were aimed at the development of thin-film light-guides (44) and luminescent solar concentrators (58), but perhaps the most fascinating has been the potential use of these materials in dye-lasers. That was the very reason for our choice of rhodamine 6G as the first trapped organic molecule (3). However, it was not until 1988 that this concept was successfully demonstrated simultaneously by research groups in Japan and in the USA. Kobayashi et al. (59) have doped rhodamine 6G and B and oxazine-4 in thin films (10-100 μm) of alumina, prepared by hydrolysis of AlCl_3 . One of the main drawbacks of dye lasers is their tendency to aggregate in aqueous solutions. The sol-gel method overcomes this difficulty (3,44) and indeed Kobayashi et al. were able to reach dye concentrations as high as 10^{-2}M without aggregation. Irradiation of the doped film with a nitrogen-laser produced laser emission with a beam divergence of 0.1 rad. It was found that the width of the dye laser emission was 10 nm, which is typical of dye lasers. The calculated conversion efficiency was 21% and it was found that the power decreases linearly with the number of shots, which is indicative of a single-photon process. Later it was found (60) that increasing the concentration of the dye increased the pumping efficiency. This dependence indicates that the doped films operate as compact dye-laser films. The behaviour of laser-dye pairs was reported in the ref. 60, as well.

Dunn, Zink, Knobbe and their colleagues achieved successful lasing activity from rhodamine 6G in SiO_2 sol-gel glass (61). Samples were pumped with the 308 nm output of a XeCl excimer laser with pulses of 10-50 mJ and the output was a blue-shifted (585.8 nm) lasing emission with FWHM of 8 nm. The output power from the gel was comparable to that obtained from a control ethanol solution. Most importantly, the pump power from the excimer laser was at levels which would bleach the dye when incorporated in an organic polymer matrix. The gain of the glass was 4.6 cm^{-1} , compared to 6.9 cm^{-1} in ethanol. In subsequent studies (48,62), this group successfully extended their studies to other laser dyes (rhodamines and coumarines) trapped in organically modified silicas where improved photostability (compared to, e.g., polymethyl methacrylate) was observed (62).

Lasing activity from sulforhodamine-640-doped silica sol-gel was recently reported by Salin et al. (63). The doped glass was pumped with a frequency-doubled radiation from a Q-switched Nd:YAG laser, and a 20%

conversion efficiency was obtained. The important aspect of that report is the 40 nm tunability of that material.

One should also mention a different approach in which laser-dyes and optically nonlinear dyes were not trapped by the sol-gel method, but adsorbed on the pores of the glass via an organic polymer solution. This approach which is outside the scope of the present review, is described in (64).

Finally, an interesting application of fluorescent sol-gel glasses is for radiation scintillating detectors. This has been described recently by Nogues et al. (65).

The incorporation of fluorescent and lasing dyes in sol-gel glasses, is perhaps the fastest growing application. After completion of this review, several additional papers on this subject were brought to our attention; these are collected in reference 65a.

4.2.2 Phosphorescent Sol-Gel Glasses (33,66). One of the most remarkable manifestations of the special properties of the silica sol-gel cage is the observation that many trapped organic molecules exhibit efficient phosphorescence at room temperature when exposed to air, in many instances without the aid of a heavy atom, and in several cases even in the wet gel. The reader probably recalls that phosphorescence is a delicate process which is usually quenched at temperatures which are not cryogenic and by exposure to oxygen. The generality of the phenomenon was demonstrated with the following molecules: Phenanthrene, naphthalene, quinine, 4-biphenyl carboxylic acid, 1-naphthoic acid, eosin-y and pyrene. It was observed that under various gelation conditions, most dyes exhibited not only phosphorescence but also delayed fluorescence. Specific glasses were needed to observe phosphorescence from the various compounds. For example, neutral gelation conditions were sufficient to observe phosphorescence from phenanthrene, naphthalene and quinine, but basic conditions were needed for the two carboxylic acids and for eosin. A heavy atom (Br) was needed to observe phosphorescence from pyrene, and so an SiO_2 glass doped with NaBr was prepared. Very long emission lifetimes were observed. For instance, when biphenylcarboxylic acid was trapped in an SiO_2 glass prepared under basic conditions, the lifetime reached the order of several seconds. Lifetimes of the order of milliseconds were obtained even from the wet gels. A detailed mechanistic study of the sol-gel transition was carried out with that carboxylic acid.

5. The Use of Trapped Molecules for Mechanistic Studies of the Sol-Gel-Xerogel Transitions and of the Cage Properties

5.1 Pyrene and its Derivatives as Probe Molecules.

The relatively long singlet lifetime of excited pyrene (≈ 100 nsec), the clear vibronic features of its fluorescence emission, its ability to form an excimer and the sensitivity of all these parameters to environmental conditions, have popularized the use of this molecule as a dopant probe for the study of sol-gel glasses (5b,33,67-71a). A brief description of some of these studies follows:

Kaufman et al. (67) performed a detailed study of the structural changes at the molecular scale that a polymerizing $\text{Si}(\text{OCH}_3)_4$ system undergoes along the sol-gel-xerogel transition. Changes in the emission spectra of pyrene and of its excimer revealed complex structural changes which proceed well beyond the gel point. The vibronic fingerprint of pyrene was used for a follow-up of polarity changes around the probe molecule, along the polymerization. Tested polymerization parameters were pH, water/silane ratio and the nature of the alkoxy group. It was found that polymerization-gelation occurs at low water/silane ratio, whereas colloidal gelation occurs at the higher water/silane ratios. A remarkable observation has been that at the final xerogel stage, no excimer emission was observed, demonstrating again the efficient trapping and isolation of doped molecules in these glasses. The excimer/monomer ratio which changes along the polymerization from ≈ 0.4 to $\approx \text{zero}$ was consequently used for the identification of isostructural gels, obtained under different reaction conditions. Matsui et al. (70) have confirmed most of these conclusions by performing a comparative study, using pyrene and pyrene-3-carboxyaldehyde for the acidic polymerization of $\text{Si}(\text{OCH}_2\text{CH}_3)_4$.

Evidence for some ground state pyrene association was provided by Yamanaka et al. (69/71) in doped SiO_2 thin films, based on time-resolved fluorescence spectroscopy.

An interesting observation, made by Kaufman et al. (described in detail in 72 and briefly in 33), has been the following: When the polymerization of $\text{Si}(\text{OCH}_3)_4$ is carried out with pyrene as a dopant and in the presence of a surface active agent, prolonged (over 1,000 hours) structural oscillations occur at the xerogel stage. The oscillations, as revealed from changes in the monomer/excimer ratio are of large amplitude, they are slow (several hrs/period) and they are chaotic in nature. The origin of this phenomenon is not clear. It has been suggested tentatively that the driving force for the oscillations is the structural relaxation of the secondary polymeric gel structure and the dispersion of the adsorbed pyrene molecule to thermodynamically favored cage sites; further exploration of this phenomenon is needed.

A pyrene derivative, 8-hydroxy-1,3,6-pyrenesulfonic acid (pyranine) was successfully used (73) for the detection of water consumption during the early stages of the polymerization of $\text{Si}(\text{OCH}_3)_4$. The idea of using this probe molecule is based on the fact that its excited state is a short-lived strong acid, capable of efficient proton transfer to neighboring water molecules, leaving behind the anion of pyranine in its excited state. Since the anion and the undissociated acid fluoresce at distinctly different wavelengths (510 and 430 nm, respectively) the ratio of intensities of the fluorescence at these two wavelengths can be used as a water-probe. Indeed, this probe showed high sensitivity to the variations in the dynamical changes of water contents (water consumption during the hydrolysis stage and water release during the condensation) as a function of variations in the polymerization conditions, i.e., changes in water/silane ratio and pH. A similar study was carried out by Pouxviel et al. on aluminosilicate sol-gels (6). Here too, pyranine proved to be a highly sensitive probe to changes in water contents of this polymerizing

system.

5.2 Various Other Probe Molecules.

The rhodamines were also used intensively to study cage properties of the glasses (3,53,74,75). Thus, the cage polarity of SiO_2 sol-gel was determined from spectral shifts of rhodamine 6G (3) and it was found that the cage is hydroxylic and highly polar, although its polarity is somewhat less than water (but higher than methanol). Fujii et al. used rhodamine B for a follow-up of the polymerization of $\text{Si}(\text{OCH}_2\text{CH}_3)_4$ (74) and found a remarkable thermal stability of that trapped dye (53). This stabilization effect of the cage seems to be general: It was found for fluorescein (46), and for trapped enzymes (27,28). A detailed study by Pouxviel et al. demonstrated that the cage electrical charge influences the trapped molecules, which in their study were rhodamine 6G and rhodamine B.

Various aromatic polycyclic molecules were employed for structural studies. Matsui et al. (76) have used 7-azaindole to study the polymerization of $\text{Si}(\text{OCH}_2\text{CH}_3)_4$ by following spectral shifts in the 380-430 nm range. It was found that 7-azaindole forms a strong complex with the silanols in the cage, stabilizing both the ground state and the excited state. Fujii et al. used 1- and 2-naphthols (77) and reported that they find these two molecules to be very convenient photoprobes. Interestingly, these authors observed an inversion of the fluorescent levels 1La and 1Lb after the gel point. Yamanaka et al. observed very short events that trapped naphthalene undergoes in SiO_2 glass (78). They used picosecond time-correlated single-photon counting techniques and observed excimer formation on the ps time scale. Finally, Levy et al. studied the room temperature phosphorescence of three aromatic polycyclic hydrocarbons: naphthalene, phenanthrene and pyrene (66) trapped in SiO_2 glasses and found that the phosphorescence efficiency increases with decrease in the number of benzene rings; this was attributed to the parallel decrease in the T_1 - S_0 gap. A mechanistic study with these and other trapped molecules led to the conclusion that phosphorescence is observable in these glasses due to strong adsorption interactions with the cage walls; due to an almost complete elimination of solvent molecules from the cage; and due to the exceptional rigidity of the final cage (66).

Indeed, the rigidity of the gelating system was the topic of a study by McKiernan et al. (79). They successfully employed the emission maximum of $\text{ReCl}(\text{CO})_3\text{bipyridine}$ blue shifts as a probe for the increasing rigidity of polymerizing aluminosilicate and silica systems. It was found that the major spectral shifts in the SiO_2 system occurred at the drying stage, while in the $\text{Al}_2\text{O}_3\text{-SiO}_2$ system it occurred at much earlier stages. This was attributed to an early cage formation in the latter system.

Fluorescence polarization of several organic dopants in a polymerizing SiO_2 sol-gel system was recently described by Winter et al. (80). Their main conclusion has been that the mobility of small and medium sized probe molecules is hardly affected by the gelation and that there is no rigidity at the microscopic level; however, this conclusion seems to be in variance with other

previously cited studies, including McKiernan's (79).

Photochromic compounds, which were already mentioned in Section 4 in the context of information recording materials, were also used for the investigation of the sol-gel-xerogel transition in $\text{Si}(\text{OCH}_3)_4$ (34). The continuous environmental changes along that transition were reflected by gradual change in the photochromic behaviour of the trapped spiropyranes. In particular, the photochromism changed at a certain point into reversed photochromism. The same approach was recently applied by Preston et al. (37) for the study of the abovementioned alumino-silicate system: Aided with spiropyran probes, these authors were able to identify four distinct stages of the gelation by following photochromism rates and spectral changes.

6. Copolymerizations

The concept of co-polymerization (instead of doping) was developed by Schmidt et al. (81). For instance, organometallic complexes of cobalt and rhodium were incorporated in SiO_2 by co-polymerization of tetraethoxy silane with tri-ethoxy silane complex ligands of these metals (82). Co-polymerizations were employed also in a recent Kodak patent (83) describing the preparation of dye-polymer/sol-gel composites. Copolymerizations aimed at achieving optical non-linearity were already mentioned in Section 4.1.2. Two additional examples are given in references 84, 85.

Acknowledgments: We are deeply indebted to our students and co-workers in this project: S. Druckman, O. Lev, D. Levy, S. Rappoport, C. Rottmann, A. Slama-Schwok, S. Shtelzer and R. Zusman. These studies are currently supported by the Materials Division of the USA RDSG-UK, the Harry Kay Foundation, the Krupp Foundation, the German BMFT Foundation and by the Israel NRC. D.A. and M.O. are members of the Farkas Center for Light Energy Conversion and of the Fritz Haber Research Center for Molecular Dynamics.

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